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Loss of Heterozygosity in DNA Mismatch Repair Genes in Human Atherosclerotic Plaques

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To detect the incidence of loss of heterozygosity (LOH) in DNA mismatch repair genes (MMR) occurring in atherosclerosis, fifty human autopsy cases of atherosclerosis were examined for LOH using 19 microsatellite markers, in three single and four tetraplex microsatellite assays. The markers used are located on or close to MMR genes. Fourteen specimens (28%) showed allelic imbalance in at least one locus. Loci hMSH2 (2p22.3–p16.1), hPMS1 (2q24.1–q32.1), and hMLH1 (3p21.32–p21.1) exhibited LOH (10, 10, and 12% respectively). We found that loss of heterozygosity on hMSH2, hPMS1, and hMLH1, occurs in atherosclerosis. The occurrence of such genomic alterations may represent important events in the development of atherosclerosis. © 2000 Academic Press

Key Words: **atherosclerosis; coronary disease; enzyme (kinetics); genetic code; sequence (DNA/RNA/prot).**

Atherosclerosis seems to be a complex trait, in which multiple genetic susceptibility loci interact with each other and the environment to produce the disease phenotype (1). The heterogeneity of the disorder and the diversity of its causes, natural histories, and clinical manifestations are difficult to explain. However, contemporary research provides some fundamental pathobiologic principles that provide a basis for understanding this multifaceted process.

A common feature of atherosclerosis is the proliferation of smooth muscle cells (2). Several lines of evidence suggest that alterations at the DNA level occur and may contribute significantly to the development of the disease. These alterations include the presence of activated transforming genes (3–5), microsatellite instability (MI) (6–8) and loss of heterozygosity (LOH) (8).

According to the features of atherosclerosis, DNA repair gene failure could be assumed. An array of DNA repair systems function in the cell, in order to avoid the effects of accumulation of DNA alterations. One of them, the DNA mismatch repair system (MMR), plays a crucial role in this process by counteracting effects caused by DNA damage, genetic recombination or replication errors. In human, two different heterodimeric complexes of MutS-related proteins (hMSH2–hMSH3 and hMSH2–hMSH6) and two different heterodimeric complexes of MutL-related proteins (hMLH1–hPMS2 and hMLH1–hPMS1) have been characterized as fundamentals for the proper function of MMR, in both base and insertion/deletion mispairing (9, 10).

In this context, we have used PCR-based microsatellite DNA analysis to investigate the probability of loss of heterozygosity (LOH, has also been termed as allelic imbalance) in MMR genes, hMSH2, hPMS1, and hMLH1, in atherosclerotic plaques. Although the detection of allelic imbalance could not alone support a full gene inactivation hypothesis, it is a direct indication of genomic instability. The frequency of its occurrence in a specific locus in concordance with a specific phenotype may have a functional significance (11, 12).

METHODS

Specimens. Fifty specimens from autopsy cases (thirty-two males and eighteen females) ranging from 55 to 77 (65.9 ± 6.3) years of age, were obtained from the Laboratory of Forensic Medicine, Medical School, University of Athens, Greece. Tissue samples from atherosclerotic plaques from aorta and normal aorta were collected during autopsies. The specimens were examined histologically and were selected so as not to be calcified or containing significant fibrous components. The tissues were frozen in liquid nitrogen immediately after excision and stored until histological examination

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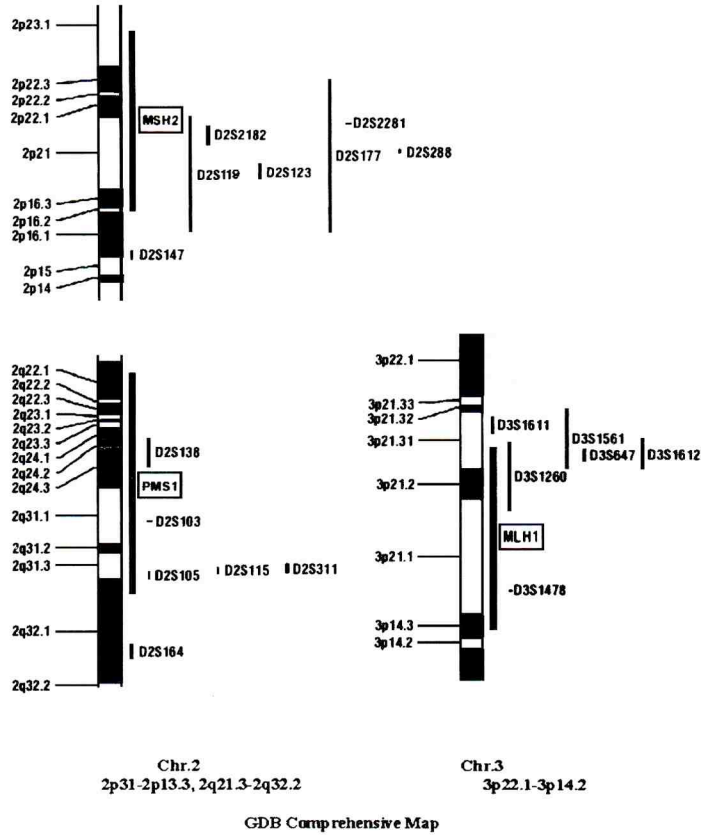

FIG. 1. Maps of the chromosomal regions where hMSH2, hPMS1, hMLH1, and DNA microsatellite markers are located.

and DNA extraction. The University of Crete ethics committee approved this study.

DNA extraction. One 0.5 cm section of each frozen sample was lysed in 400 mM Tris–HCl pH 8.0, 150 mM NaCl, 60 mM EDTA, 1% SDS, 100 μg/ml Proteinase K and incubated at 42°C for 16 h in an orbital shaker. Deproteinization included extraction with phenol/chloroform and chloroform. DNA was precipitated by the addition of an equal volume of isopropanol. DNA was washed with 70% ethanol and resuspended in 50 μl TE (10 mM Tris–HCl pH 8.0, 1 mM EDTA). Working stocks were prepared by tenfold dilution in double distilled H_2O.

Primers and PCR amplification of microsatellite loci. Nineteen microsatellite DNA markers (Research Genetics, USA) were selected, located in chromosomal regions: 2p22.3–p16.1, 2q24.1–q32.1 and 3p21.32–p21.1. These regions were chosen because hMSH2, hPMS1, and hMLH1 genes are located in these regions, respectively (Fig. 1, data from GenomeDatabase, http://gdbwww.gdb.org). Microsatellite DNA markers were amplified in four panels of 4-plex reactions and three single PCR assays (Table 1). We introduced 100 ng of genomic DNA in a PCR reaction mixture containing 1× GIBCO BRL PCR Buffer, 350 μM dNTPs, 2.66 mM $MgCl_2$ and 0.35 U GIBCO BRL *Taq* DNA polymerase (GIBCO BRL, Life Technologies). To optimize 4-plex reactions, different concentrations of each marker primer set were used (Table 1). Amplification parameters were the following: initial denaturation for 3 min; 30 cycles consisted of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s; final extension step at 72°C for 10 min. PCR products were electrophoresed in a 10% polyacrylamide gel and silver stained. LOH were scored when a significant reduction in the intensity of one allele in the heterozygous specimen was observed in the DNA of the atherosclerotic specimen. The analysis in the LOH positive cases was repeated thrice and the results were reproducible.

RESULTS

We assayed 50 atherosclerotic/normal DNA pairs from aorta with a total of 19 microsatellite markers. The incidence of LOH for each marker ranged from 0 to 5.9%, while the degree of heterozygosity ranged from 0.62 to 0.98 (Table 1). Characteristic examples of allelic imbalance are shown in Fig. 2. Fourteen (28%) showed LOH in at least one, and four of them (8%) at two of the examined loci. Allelic imbalance was most frequently observed in D3S1561 (5.9%) and D2S2291 (5.7%) loci. Homozygote genotype of a sample in a microsatellite DNA marker was considered to be noninformative in LOH analysis.

The highest incidences of LOH were 12.5% in a 69-year-old woman, 12.5% in a 72-year-old male, 11.8% in a 70-year-old woman, and 11.1% in a 55-year-old male. These samples were affected in two different genetic loci and two of them showed LOH in two different chromosomal regions (hMSH2 and PMS1, hMSH2 and hMLH1). The fractional regional loss (FRL) values were calculated for each locus as [loci on the arm with allelic imbalance]/[total informative loci on the arm] (13) and were 0.021, 0.021, and 0.026, for 2p22.3–p16.1 (hMSH2), 2q24.1–q32.1 (hPMS1), and 3p21.32–p21.1 (hMLH1) locus, respectively.

Microsatellite alterations (MA) were not observed in any locus since no novel generated microsatellite allele was observed in atherosclerotic DNA, compared to the normal sample examined.

DISCUSSION

Atherosclerosis is not merely a disease in its own right, but a process that is the principal contributor to the pathogenesis of coronary heart disease and/or cerebral and peripheral vascular disease (2). This spectrum implies a complex trait, where the importance of inflammation, smooth muscle and monocyte replication, connective tissue formation, and lipid accumulation is well established. The aetiology and pathogenesis of the disease are tightly linked with the ubiquitous

TABLE I

Microsatellite DNA Markers Studied

Locus	Cytogenetic location	Het[a]	ASR[b] (bp)	FRL[c]	Proximal genes	Primer concentration in reaction (nM)	Panel[d]
D2S119	2p22.3–p16.1	0.80	214–232	0.023	hMSH2	135	A
D2S123	2p22.3–p16.1	0.76	196	0.024	hMSH2	120	A
D2S177	2p22.3–p16.1	0.85	276–302	0	hMSH2	147	A
D2S147	2p22.3–p16.1	0.73	126–144	0.047	hMSH2	100	A
D2S2182	2p22.3–p16.1	0.78	234	0.024	hMSH2	136	B
D2S288	2p22.3–p16.1	0.62	276–284	0.028	hMSH2	145	B
D2S2291	2p22.3–p16.1	0.76	245	0	hMSH2	120	C
D2S138	2q24.1–q32.1	0.67	111–125	0.029	hPMS1	100	C
D2S103	2q24.1–q32.1	0.82	109–125	0.023	hPMS1	100	—[e]
D2S105	2q24.1–q32.1	0.69	107–125	0.054	hPMS1	105	D
D2S115	2q24.1–q32.1	0.71	106–126	0	hPMS1	100	—[e]
D2S164	2q24.1–q32.1	0.83	265–303	0.045	hPMS1	147	C
D2S311	2q24.1–q32.1	0.81	185–207	0	hPMS1	120	D
D3S1611	3p21.32–p21.1	0.66	252–268	0.057	hMLH1	138	C
D3S1260	3p21.32–p21.1	0.66	268	0	hMLH1	140	D
D3S1561	3p21.32–p21.1	0.65	226	0.059	hMLH1	132	D
D3S1612	3p21.32–p21.1	0.69	100	0.026	hMLH1	80	B
D3S647	3p21.32–p21.1	0.73	100	0	hMLH1	80	—[e]
D3S1478	3p21.32–p21.1	0.98	109–152	0.020	hMLH1	120	B

[a] Het, observed heterozygosity.
[b] ASR, allele size range.
[c] FRL, fraction regional loss.
[d] Panel of 4-plex reaction.
[e] Single PCR assay.

protective mechanisms associated with inflammation and repair. Subcellular alterations include changes in the levels of molecules involved in mitogenic signalling and genetic aberrations (14, 15). The latter leading to the assumption that DNA repair genes could also be affected during this process.

Three pathways have been proposed for the full inactivation of a gene allele: intragenic mutations, loss of chromosomal material (allelic imbalance or homozygous deletion) and DNA methylation of CpG islands located in the promoter of the gene (11). In this study we used allelic imbalance in order to examine the integrity of three basic genes for the proper function of the MMR system.

The MMR, which is involved in increasing the fidelity of replication by specific repair of DNA polymerase incorporation errors, was discovered earlier in prokaryotes, but has been shown to be involved in cancer only within past five years. Mutations in mismatch repair genes leads to cancer predisposition (16). Loss of function of MMR genes creates a characteristic mutator phenotype known as microsatellite instability (MIN) (17, 18). Tumors exhibiting MIN lacked detectable MMR activity in biochemical assays (19, 20).

We used microsatellite DNA assays, to identify genetic alterations in MMR fundamental genes, hMSH2, hPMS1 and hMLH1. Our results demonstrated the

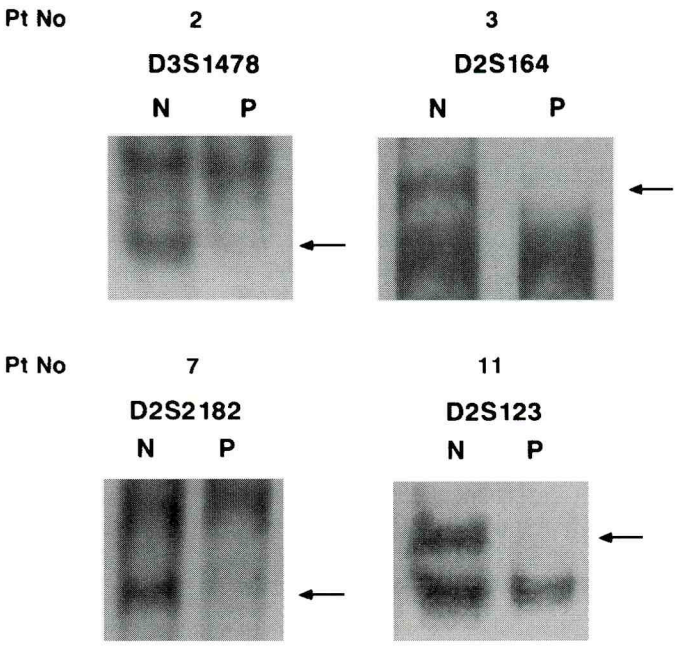

FIG. 2. Representative examples of specimens exhibiting LOH. P, atherosclerotic plaque. N, normal tissue. Arrows indicate the position of a deleted allele. The faint bands in the position of the deleted alleles are interpreted as contamination by the adjacent normal tissue. The numbers above the locus name represent the patient numbers.

incidence of LOH on 2p22.3–p16.1, 2q24.1–q32.1, and 3p21.32–p21.1, as 10, 10, and 12%, respectively. These findings suggest the implication of these loci in atherosclerosis. Although it is not possible to determine whether these genomic aberrations are involved in the pathophysiologic events of the atherosclerotic process, their presence reveals that they should be consider as factors implicated in atherosclerosis.

Although the incidence of LOH in these chromosomal regions, reported in the present investigation is not very high, the total or partial deletion of one allele of these genes might lead to reduction of DNA repair capacity. This study gives new information on candidate susceptibility loci involved in atherosclerosis.

In conclusion, we have detected a considerable incidence of LOH in MMR genes in aortic atherosclerotic lesions indicating the presence of a decreased fidelity in DNA repair in atherosclerotic tissues. Although the clinical significance of this observation remains unknown, such genetic alterations reflect an extensive genomic instability of atherosclerotic plaques and may represent an additional factor involved in pathogenesis of atherosclerosis.

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